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Incidence and synergism of resistance to conventional insecticides in larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), in northern Xinjiang, China

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Abstract: To understand the resistance levels and dynamics of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (CPB) in Xinjiang to conventional insecticides and offer the fundamental data for resistance management, six overwintered CPB adult populations were collected from potato fields of Urumqi, Fukang, Changji, Altay, Nilka, and Tekes counties (cities) in northern Xinjiang Uygur autonomous region in China, and the first generation of 4th instar larvae were tested for resistance to fourteen conventional insecticides by topical application. And possible resistance mechanisms were investigated using three synergists, triphenyl phosphate (TPP), diethylmeleate (DEM), and piperonyl butoxide (PBO). The results showed that the Tekes field population was susceptible to several tested insecticides when compared to the baselines that have been documented. This population, therefore, was selected as a reference susceptible strain. Compared with the Tekes strain, Changji and Nilka populations had extremely high- to high- or moderate-level resistance to cyhalothrin (resistance ratio was 108.8- and 29.4-fold, respectively), deltamethrin (30- and 42.5-fold, respectively), carbofuran (17.6 and 10.1-fold, respectively), and carbosulfan (24.7- and 89.9-fold, respectively). Moreover, the Changji and Fukang beetles developed low- to moderate-level resistance to α-cypermethrin (8.9- and 13.0-fold, respectively). Synergism studies revealed that PBO had significant synergism to cyhalothrin in the Nilka population, and PBO, TPP and DEM exhibited significant synergism to carbofuran in the Changji population. These results suggest that enzymatic detoxification may be one of the resistance mechanisms to cyhalothrin and carbofuran for local populations in Xinjiang. Because the synergists do not completely eliminate CPB resistance, there are probably additional resistance mechanisms involved.

Key words: Colorado potato beetle; insecticide resistance; topical bioassay; synergist; synergism; resistance mechanism

1 INTRODUCTION

Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), native to the southwestern United States and Mexico, has become the most important insect defoliator of potatoes throughout North America, Europe, and parts of Asia (Alyokhin *et al*., 2008; Alyokhin, 2009). The beetle invaded China in the 1990s from Kazakhstan, and finally established in Huocheng, Qapqal, and Yining counties, and Taicheng city in Xinjiang Uygur autonomous region in 1992. Since then, it has spread eastward. The beetle is currently known to be widely distributed through most of northern Xinjiang, including Urumqi city, Qitai, Jimsar and Mori Kazak autonomous counties.

CPB causes significant damage to potato and eggplant in northern Xinjiang. The beetles consume potato plant leaves, causing a 30%-50% yield reduction each year. A larva consumes an average of 40 cm^2 of potato leaves before it pupates, and as the adult, the beetle causes nearly 10 cm^2 of foliage

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damage per day. Once the foliage is gone, the beetles can feed on stems and exposed tubers. If left uncontrolled, the beetles can completely destroy potato crops. In addition to impressive feeding rates, CPB is also characterized by high potential fecundity, with one female laying about 600 eggs on average (Alyokhin *et al.*, 2008; Alyokhin, 2009).

Chemical control is widely practiced. Since 1864, hundreds of compounds have been tested against CPB. Currently, insecticides still remain a very important component of a management program for effective control of CPB on commercial potato farms (Barčić *et al.*, 2006; Baker *et al.*, 2008; Alyokhin, 2009). More than 30 active ingredients, such as cyclodiene organochlorines, carbamates, organophosphates, pyrethroids, pyrethrins, neonicotinoids, spinosyns, avermectins, cryolites, *Bacillus thuringiensis* subsp. *tenebrionis* endotoxins, benzoylureas, azadirachtins, indoxacarbs, and bifenazates, are registered for use against CPB around the world (Alyokhin *et al.*, 2008).

Not surprisingly, insecticide selection pressure eventually resulted in the development of insecticide resistance. The first example of CPB resistance to synthetic organic pesticides was documented for DDT in 1952 (Alyokhin *et al.*, 2008). Since then, this insect has developed resistance to every class of insecticides, including the arsenicals, organochlorines, carbamates, organophosphates, pyrethroids and even neonicotinoids worldwide (Cutkomp *et al.*, 1958; Harris and Svec, 1976; Johnston and Sandvol, 1986; Ioannidis *et al.*, 1991; French *et al.*, 1992; Mota-Sanchez *et al.*, 2000; Noronha and Goettel, 2002; Leontieva *et al.*, 2006; Mota-Sanchez *et al.*, 2006; Baker *et al.*, 2007; Alyokhin *et al.*, 2008; Ben'kovskaya *et al.*, 2008). Therefore, it is urgent and essential to estimate insecticide resistance in CPB in northern Xinjiang Uygur autonomous region for proper choice of insecticides.

2 MATERIALS AND METHODS

2.1 Insects

Potato was a single-season crop growing from May to August or September in Xinjiang Uygur autonomous region, China. The overwintered CPB adults emerged and fed on the leaves of potato plants in the early to mid-May. Six overwintered-adult samples from Urumqi city (43.82°N, 87.61°E, 2008), Fukang county (44.08°N, 87.91°E, 2009), Changji city (44.01°N, 87.25°E, 2009), Altay city (47.50°N, 88.08°E, 2009), Nilka county (43.79°N, 82.50°E, 2009), and Tekes county (43.22°N, 81.84°E, 2009) in northern Xinjiang were collected from potato fields in 2008 and 2009. The rearing conditions of CPB adults were 28 ± 1℃, photoperiod 14L:10D, 50%-60% RH, and fresh potato foliage being as food. The 4th instar larvae of the 1st generation beetles were used to monitor insecticide resistance.

2.2 Chemicals

The technical-grade active ingredients of five pyrethroids (bifenthrin, cyhalothrin, α-cypermethrin, deltamethrin and permethrin), three carbamates (carbaryl, carbofuran and carbosulfan), five organophosphates (azinphosmethyl, diazinon, isocarbophos, malathion and phoxim), and an organochlorine (endosulfan) were used in the present study (Table 1).

Table 1 Details of fourteen technical grade insecticides

Insecticide	Purity (%)	Manufacturer
Bifenthrin	97.0	Jiangsu Yangnong Chemical Group Co., Ltd)
Cyhalothrin	95.0	Nanjing Red Sun Group Limited Company
α-Cypermethrin	95.0	Haili Chemical company limited by shares
Deltamethrin	98.0	Nanjing Red Sun Group Limited Company
Permethrin	93.0	Jiangsu Nanjing Aijin Chemical Group Limited Company
Azinphosmethyl	98.9	Sigma-Aldrich
Diazinon	95.0	Nanjing Hongliyuan Chemical Industry
Isocarbophos	98.4	Nanjing Red Sun Group Limited Company
Malathion	95.0	Nanjing Red Sun Group Limited Company
Phoxim	90.0	Jiangsu Tongzhou Zhengda Pesticide Factory
Carbayl	99.0	Haili Guixi Chemical Industry Pesticide Factory
Carbofuran	97.0	Nanjing Red Sun Group Limited Company
Carbosulfan	90.0	Nanjing Red Sun Group Limited Company
Endosulfan	94.5	Jiangsu Nanjing Aijin Chemical Group Limited Company

Triphenyl phosphate (TPP) was obtained from Beijing Chemical Reagent in China. Diethylmeleate (DEM) was purchased from Shanghai Chemical Reagent in China, and piperonyl butoxide (PBO) was acquired from Sigma. Acetone was from Nanjing Chemical Reagent Co., Ltd in China. All belonged to the analytical grade. These chemicals were kept in a refrigerator between the experimental sessions.

2.3 Bioassays

Topical bioassays were used to assess CPB susceptibilities to the insecticides. Technical-grade active ingredients were dissolved in analytical-grade acetone and at least five concentrations that resulted in more than 0% and less than 100% mortality based on preliminary assays were used for each of the insecticides. Ten 4th instar larvae were treated individually with 0.22 μL of insecticide solution applied by a 10 μL microsyringe connected to a microapplicator (Hamilton Company, Reno, NV) to the 4th dorsal abdominal segment. Each control larva received 0.22 μL of acetone. Control mortality was typically less than 10%. Three to five replications per concentration were performed.

The synergistic effects of TPP, DEM and PBO on several insecticides were estimated. TPP, DEM and PBO were normally considered as the inhibitor of esterases, glutathione S-transferase and cytochrome P450 monooxygenases, respectively. These chemicals were dissolved in analytical-grade acetone and applied individually to the 4th dorsal abdominal segment 1 h prior to insecticide application at a dose of 1 μg per larva, an amount having no obvious influence on the larvae in a preliminary test. Acetone was applied as the control.

After treatment, larvae were placed in Petri dishes (9 cm in diameter and 1.5 cm in height) containing fresh potato leaves and kept at 28℃, 50%-60% RH, and 14L∶10D photoperiod. The insecticides in the present paper could rapidly kill CPB larvae, mortalities were assessed 24 h later. The treated larvae were considered as alive if they could move their legs and body after touching one leg with a fine needle, a criterion presented by Sharif *et al.* (2007).

2.4 Data statistics and analysis

Abbott's formula was used to correct the data for control mortality. Probit analysis was used to estimate the doses needed to cause 50% mortality (LD_{50}), their fiducial limits, and the slope of the line relating probit mortality to the log dose. Resistance ratio (RR) was determined by comparing the LD_{50} value of each local population to each of the insecticides with the corresponding LD_{50} value of a reference population. Synergism ratio was calculated by dividing the LD_{50} value of unsynergized treatment with the LD_{50} value of synergized treatment.

3 RESULTS

3.1 The Tekes field population as a susceptible strain

The susceptibilities of 4th instar larvae of the Tekes field population to fourteen conventional insecticides were evaluated by a topical bioassay. The LD_{50} values of azinphosmethyl, carbofuran, and endosulfan were 3.33×10^{-1}, 2.33×10^{-2}, and 1.27×10^{-1} μg per larva, respectively (Table 2), completely comparable to those to some susceptible strains (Rose and Brindley, 1985; Argentine *et al.*, 1989a; Sharif *et al.*, 2007). Therefore, we selected the Tekes field population as an insecticide susceptible reference strain.

3.2 Susceptibility of 4th instar larvae

Compared with the Tekes susceptible reference strain, the Changji and Nilka populations developed 108.8- and 29.4-fold resistance to cyhalothrin, 30- and 42.5-fold resistance to deltamethrin, respectively. Similarly, the Changji and Fukang beetles developed 8.9- and 13.0-fold resistance to α-cypermethrin, respectively. However, the Urumqi, Altay and Fukang populations were still susceptible to these three pyrethroids. The Urumqi population was susceptible to bifenthrin and permethrin (Table 2).

Among the tested field populations, the Changji population developed 17.6- and 24.7-fold resistance to carbofuran and carbosulfan, respectively, and the Nilka larvae developed 10.1- and 89.9-fold resistance to carbofuran and carbosulfan, respectively compared with the Tekes strain. All these field populations, however, were susceptible to carbayl (Table 2). All the tested field populations remained susceptible to five organophosphates and one cyclodiene organochlorine (Table 2).

3.3 Synergistic effects of several enzyme inhibitors

The synergisms of PBO, TPP and DEM on cyhalothrin in the Tekes, Changji and Nilka populations were tested. All the three synergists significantly enhanced the toxicities of cyhalothrin in the Tekes population. Moreover, PBO showed great synergistic effect on the Nilka population. However, these three synergists only had slight or no synergistic effects in the Changji beetles (Table 3).

Table 2 Toxicities of fourteen conventional insecticides to 4th instar larvae of *Leptinotarsa decemlineata*

Insecticides	Population	Number of tested individuals	Slope (*b*)	LD_{50} (μg/larva) (95% FL)	RR
Bifenthrin	Tekes	212	1.8375	$1.50\times10^{-3}(9.44\times10^{-4}-2.11\times10^{-3})$	1.0
	Urumqi	180	2.0488	$6.11\times10^{-3}(3.04\times10^{-3}-1.24\times10^{-2})$	4.1
Cyhalothrin	Tekes	240	0.8305	$5.01\times10^{-4}(1.04\times10^{-4}-1.95\times10^{-3})$	1.0
	Changji	182	0.5444	$5.44\times10^{-2}(2.06\times10^{-2}-1.43\times10^{-1})$	108.8
	Nilka	183	1.5573	$1.47\times10^{-2}(3.64\times10^{-3}-5.98\times10^{-2})$	29.4
	Fukang	212	2.5894	$1.01\times10^{-3}(7.04\times10^{-4}-1.50\times10^{-3})$	2.0
	Altay	241	1.1875	$6.04\times10^{-4}(4.04\times10^{-4}-1.01\times10^{-3})$	1.2
	Urumqi	183	1.3189	$1.46\times10^{-4}(7.40\times10^{-5}-3.02\times10^{-4})$	0.2
α-Cypermethrin	Tekes	211	0.9524	$2.70\times10^{-3}(3.04\times10^{-4}-2.19\times10^{-2})$	1.0
	Changji	240	1.2057	$2.40\times10^{-2}(1.09\times10^{-2}-5.32\times10^{-2})$	8.9
	Nilka	180	1.2131	$1.14\times10^{-2}(4.04\times10^{-3}-3.26\times10^{-2})$	4.2
	Fukang	210	0.8745	$3.50\times10^{-2}(4.70\times10^{-3}-2.60\times10^{-1})$	13.0
Deltamethrin	Tekes	185	0.5362	$2.01\times10^{-4}(1.03\times10^{-4}-3.64\times10^{-3})$	1.0
	Changji	212	2.2213	$6.03\times10^{-3}(3.84\times10^{-3}-9.61\times10^{-3})$	30
	Nilka	240	0.6932	$8.51\times10^{-3}(1.04\times10^{-4}-1.74\times10^{-2})$	42.5
Permethrin	Tekes	182	2.4232	$2.83\times10^{-2}(1.43\times10^{-2}-4.25\times10^{-2})$	1.0
	Urumqi	210	1.2904	$1.87\times10^{-2}(4.34\times10^{-3}-8.18\times10^{-2})$	0.7
Carbayl	Tekes	183	3.1778	$1.98\times10^{1}(1.03\times10^{1}-3.82\times10^{1})$	1.0
	Changji	213	3.0236	$1.34\times10^{1}(8.45-2.11\times10^{1})$	0.7
	Nilka	240	2.3904	$7.70\ (1.68-3.51\times10^{1})$	0.4
Carbofuran	Tekes	180	1.4989	$2.33\times10^{-2}(1.44\times10^{-3}-3.76\times10^{-1})$	1.0
	Changji	214	2.9722	$4.11\times10^{-1}(2.55\times10^{-1}-6.62\times10^{-1})$	17.6
	Nilka	242	3.2264	$2.35\times10^{-1}(1.40\times10^{-1}-3.96\times10^{-1})$	10.1
	Urumqi	180	0.8555	$8.30\times10^{-3}(1.24\times10^{-3}-5.85\times10^{-2})$	0.4
Carbosulfan	Tekes	241	1.8167	$1.12\times10^{-2}(5.74\times10^{-3}-2.19\times10^{-2})$	1.0
	Changji	272	3.2609	$2.77\times10^{-1}(1.94\times10^{-1}-3.96\times10^{-1})$	24.7
	Nilka	184	3.5317	$1.01\ (6.22\times10^{-1}-1.63)$	89.9
Azinphosmethyl	Tekes	180	1.3898	$3.33\times10^{-1}(1.48\times10^{-1}-7.48\times10^{-1})$	1.0
	Changji	240	2.9373	$1.86\ (1.51-2.29)$	5.6
	Nilka	211	3.2545	$9.95\times10^{-1}(5.71\times10^{-1}-1.74)$	3.0
Diazinon	Tekes	184	1.9995	$2.49\ (1.29-4.81)$	1.0
	Changji	211	2.7433	$9.49\ (5.46-1.65\times10^{1})$	3.8
	Nilka	241	2.8278	$6.26\ (4.44-8.82)$	2.5
Isocarbophos	Tekes	180	3.1740	$6.50\times10^{-1}(3.69\times10^{-1}-1.14)$	1.0
	Changji	213	2.5321	$1.07\ (6.56\times10^{-1}-1.73)$	1.6
	Nilka	245	2.4156	$1.18\ (5.82\times10^{-1}-2.41)$	1.8
	Urumqi	210	1.0240	$8.19\times10^{-2}(2.41\times10^{-2}-2.78\times10^{-1})$	0.1
Malathion	Tekes	214	3.1093	$9.28\times10^{1}(5.35\times10^{1}-1.6\times10^{2})$	1.0
	Changji	240	2.2723	$4.09\times10^{1}(9.90\times10^{-1}-1.69\times10^{2})$	0.4
	Nilka	185	1.0475	$1.53\times10^{2}(8.06\times10^{1}-2.90\times10^{2})$	1.6
Phoxim	Tekes	211	3.6218	$1.77\times10^{-1}(9.37\times10^{-2}-3.34\times10^{-1})$	1.0
	Changji	184	3.6489	$1.44\times10^{-1}(9.46\times10^{-2}-2.21\times10^{-1})$	0.8
	Nilka	244	3.0839	$1.55\times10^{-1}(7.15\times10^{-2}-3.37\times10^{-1})$	0.9
Endosulfan	Tekes	182	1.8887	$1.27\times10^{-1}(5.36\times10^{-2}-2.99\times10^{-1})$	1.0
	Changji	210	2.1468	$9.46\times10^{-2}(6.07\times10^{-2}-1.47\times10^{-1})$	0.7
	Nilka	181	1.1725	$2.04\times10^{-1}(6.18\times10^{-2}-6.74\times10^{-1})$	1.6
	Urumqi	212	1.9154	$7.37\times10^{-2}(3.68\times10^{-2}-1.48\times10^{-1})$	0.6

LD_{50} is the dose that causes 50% mortality, and FL represents the fiducial limit of LD_{50} at $P=0.05$. The same below.

Table 3 Synergistic effects of several enzyme inhibitors on cyhalothrin in the 4th instar larvae of *Leptinotarsa decemlineata*

Population	Synergistic treatment	Number of tested individuals	LD_{50} (μg/larva) (95% FL)	Synergistic ratio
Tekes	Acetone	240	$5.01 \times 10^{-4}(1.04 \times 10^{-4} - 1.95 \times 10^{-3})$	
	PBO	271	$1.00 \times 10^{-4}(1.04 \times 10^{-5} - 4.13 \times 10^{-3})$	5
	DEM	242	$1.02 \times 10^{-4}(1.03 \times 10^{-5} - 4.50 \times 10^{-3})$	5
	TPP	213	$4.02 \times 10^{-5}(1.02 \times 10^{-5} - 1.66 \times 10^{-3})$	12.5
Changji	Acetone	182	$5.44 \times 10^{-2}(2.06 \times 10^{-2} - 1.43 \times 10^{-1})$	
	PBO	271	$2.31 \times 10^{-2}(4.54 \times 10^{-2} - 1.18 \times 10^{-1})$	2.4
	DEM	240	$1.57 \times 10^{-1}(2.16 \times 10^{-2} - 1.15 \times 10^{-1})$	0.3
	TPP	210	$6.68 \times 10^{-2}(1.26 \times 10^{-2} - 3.53 \times 10^{-1})$	0.8
Nilka	Acetone	183	$1.47 \times 10^{-2}(3.64 \times 10^{-3} - 5.98 \times 10^{-2})$	
	PBO	270	$1.43 \times 10^{-3}(2.01 \times 10^{-4} - 1.05 \times 10^{-2})$	10.5
	DEM	183	$7.01 \times 10^{-3}(2.74 \times 10^{-3} - 1.81 \times 10^{-2})$	2.1
	TPP	213	$4.11 \times 10^{-3}(3.04 \times 10^{-4} - 6.30 \times 10^{-2})$	3.6

Table 4 Synergistic effects of several enzyme inhibitors on carbofuran in the 4th instar larvae of *Leptinotarsa decemlineata*

Population	Synergistic treatment	Number of tested individuals	LD_{50} (μg/larva) (95% FL)	Synergistic ratio
Tekes	Acetone	180	$2.33 \times 10^{-2}(1.44 \times 10^{-3} - 3.76 \times 10^{-1})$	
	PBO	182	$2.79 \times 10^{-2}(6.53 \times 10^{-3} - 1.20 \times 10^{-1})$	0.8
	DEM	213	$3.45 \times 10^{-2}(1.26 \times 10^{-2} - 9.46 \times 10^{-2})$	0.7
	TPP	240	$2.83 \times 10^{-2}(2.20 \times 10^{-3} - 3.63 \times 10^{-1})$	0.8
Changji	Acetone	214	$4.11 \times 10^{-1}(2.55 \times 10^{-1} - 6.62 \times 10^{-1})$	
	PBO	212	$3.1 \times 10^{-3}(1.64 \times 10^{-3} - 5.81 \times 10^{-1})$	132.4
	DEM	238	$7.79 \times 10^{-2}(3.03 \times 10^{-2} - 2.01 \times 10^{-1})$	5.3
	TPP	229	$7.05 \times 10^{-2}(1.64 \times 10^{-2} - 3.04 \times 10^{-1})$	5.8
Nilka	Acetone	242	$2.35 \times 10^{-1}(1.40 \times 10^{-1} - 3.96 \times 10^{-1})$	
	PBO	232	$5.50 \times 10^{-2}(1.61 \times 10^{-2} - 1.87 \times 10^{-1})$	4.3
	DEM	191	$1.52 \times 10^{-1}(8.61 \times 10^{-2} - 2.67 \times 10^{-1})$	1.5
	TPP	239	$7.39 \times 10^{-2}(2.87 \times 10^{-2} - 1.90 \times 10^{-1})$	3.2

For carbofuran, PBO, TPP and DEM did not show synergistic effect in the Tekes population. However, all the three synergists exhibited greater synergistic effects in the Changji population and showed some synergistic effects in the Nilka population. Among the three synergists, PBO enhanced the toxicity the most, followed by TPP, and then DEM (Table 4).

4 DISCUSSION

4.1 Tekes field population as the susceptible reference strain

In the present paper, topical bioassays were used to evaluate the susceptibilities of the 4th instar larvae of the Tekes field population to fourteen conventional insecticides. The LD_{50} values of azinphosmethyl, carbofuran, and endosulfan were 3.33×10^{-1}, 2.33×10^{-2}, and 1.27×10^{-1} μg per larva, respectively. It has been reported that the LD_{50} value of azinphosmethyl to Raleigh susceptible strain was 2×10^{-1} μg per larva (Argentine *et al.*, 1989b), of carbofuran was 5.8×10^{-1} μg per larva (Rose and Brindley, 1985), and of endosulfan to Lethbridge susceptible strain was 1×10^{-1} μg per larva (Sharif *et al.*, 2007). Our results demonstrated that the susceptibilities of the Tekes field population to these three insecticides were completely comparable to those to some susceptible strains. Consistent with this, Tekes county is located in the Tekes steppes in the northern side of Tianshan Mountains at about 1 500 m above sea level. The climate is cool and the frost-free growing season is short. Farmers always use insecticide-free strategies to control agricultural pests. Therefore, we selected the Tekes field population as an insecticide susceptible reference strain.

4.2 Resistance status of Colorado potato beetle in Xinjiang

Compared with the Tekes susceptible strain, the field populations from Changji city and Nilka county developed 108.8- and 29.4-fold resistance to cyhalothrin, 30- and 42.5-fold resistance to deltamethrin, 17.6- and 10.1-fold resistance to carbofuran, and 24.7- and 89.9-fold resistance to carbosulfan, respectively. Moreover, the field populations from Changji city and Fukang county developed 8.9- and 13.0-fold resistance to α-cypermethrin, respectively. These results demonstrated that the CPB in Xinjiang had developed low- to high-level resistance to pyrethroids (cyhalothrin, α-cypermethrin, deltamethrin), and two carbamates (carbofuran and carbosulfan). Consistent with our results, CPB has a legendary ability to develop resistance very quickly to virtually every insecticide used against it in response to local selection (Mota-Sanchez *et al.*, 2006; Alyokhin *et al.*, 2008; Ben' kovskaya *et al.*, 2008; Alyokhin, 2009). Some of CPB larvae populations from Idaho, for example, had low susceptibility to fenvalerate, low-level resistance to azinphosmethyl, moderate-level resistance to aldicarb (Johnston and Sandvol, 1986), and some of CPB larvae populations from Idaho or Iran developed moderate- or high-level resistance to endosulfan (Johnston and Sandvol, 1986; Sharif *et al.*, 2007). Similarly, Massachusetts resistant strain showed 55-fold resistance to permethrin (Argentine *et al.*, 1989a). Moreover, many CPB populations had developed resistance to esfenvalerate, some of which had more than 400-fold differences (Huang *et al.*, 1995). In addition, Stankovic *et al.* (2004) also found that some field populations exhibited resistance to chlorpyriphos and carbosulfan, with resistance ratios (RRs) ranging from 1.0 to 109.7, and from 1.0 to 52.8, respectively.

4.3 Resistance mechanism to cyhalothrin and carbofuran of Colorado potato beetle in Xinjiang

In the present study, the involvements of several detoxification enzymes in cyhalothrin and carbofuran metabolism were investigated by comparing the toxicities of these insecticides to 4th instar larvae pretreated with each of different enzyme inhibitors with those pretreated with acetone. The degree of synergism by these enzyme inhibitors can suggest a certain detoxification enzyme or enzymes are involved in the metabolism of pesticides and insecticide resistance. In the present paper, we used three synergists (TPP, an esterase inhibitor; PBO, a cytochrome P450-dependent monooxygenase inhibitor; and DEM, a glutathione S-transferase inhibitor) to evaluate whether these synergists could enhance the toxicity of cyhalothrin or carbofuran. For cyhalothrin, all three synergists significantly enhanced the toxicities in the Tekes population. Moreover, PBO showed great synergistic effect in the Nilka population. However, these three synergists only had slight or no synergistic effects in the Changji beetles. For carbofuran, PBO, TPP and DEM exhibited greater synergistic effects in the Changji population and showed some synergistic effects in the Nilka population. These results demonstrated that mixed-function oxidase mediated detoxification of cyhalothrin in the Nilka population, and mixed-function oxidase, esterase and glutathione S-transferase mediated detoxification of carbofuran in the Changji population. Therefore, enzymatic detoxification may be one of the resistance mechanisms to cyhalothrin and carbofuran for Xinjiang field populations.

Similarly, it was found that these detoxification enzymes were associated with insecticide resistance in CPB (Rose and Brindley, 1985; Argentine *et al.*, 1989a, 1992, 1994; Zhao *et al.*, 2000; Sharif *et al.*, 2007). Monooxygenase, for example, was involved in carbofuran metabolism in high-level carbamate resistant CPB strain (Rose and Brindley, 1985). Likewise, monooxygenase, carboxylesterase and glutathione S-transferase have been confirmed to be the mechanism of resistance to permethrin in CPB (Argentine *et al.*, 1989a; Ahammadsahib *et al.*, 1994; Argentine *et al.*, 1995; Lee and Clark, 1996). Moreover, monooxygenase and glutathione S-transferase were found to be associated with azinphosmethyl resistance in CPB (Argentine *et al.*, 1989a).

In the present study, the synergists had little synergistic effect on cyhalothrin in the Changji population and did not completely eliminate CPB resistance of the Nilka population to cyhalothrin and carbofuran, suggesting that there are probably additional mechanisms involved. In fact, target site insensitivity has been reported as an important mechanism of CPB resistance to insecticides (Alyokhin *et al.*, 2008). Altered acetylcholine esterase, for example, also plays a critical role in resistance to organophosphates and carbamates (Ioannidis *et al*., 1992; Wierenga and Hollingworth, 1993; Clark, 1997; Stanković *et al*., 2004; Kim *et al*., 2006, 2007). Similarly, an amino acid change (leucine to phenylalanine, L1014F) in an α-subunit of the sodium channel resulted in the resistance to permethrin in a CPB strain (Lee *et al*., 1999; Kim *et al*., 2005). Insensitivity at the target site as a mechanism of CPB resistance to imidacloprid has also been suggested (Tan *et al*., 2008). Further researches on corresponding target site differences between susceptible and resistant strains will shed light on the effects of target site insensitivity on insecticide resistances in the CPB field populations from northern Xinjiang Uygur autonomous region.

4.4 Suggestions for persistant control of Colorado potato beetle in Xinjiang

Reducing insecticidal pressure on pest populations is a common strategy to delay evolution of resistance (Alyokhin *et al*., 2008; Alyokhin, 2009). Selection towards insecticide resistance can be alleviated with non-chemical control methods. Some alternative control strategies such as crop rotation, manipulation of planting time, use of trap crops, plastic-lined trenches along field borders, and destruction of overwintering habitats, as well as classical biological control, have been proven to be effective (Alyokhin *et al*., 2008) and should be practiced in potato culture system in northern Xinjiang Uygur autonomous region.

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新疆北部马铃薯甲虫幼虫对常规杀虫剂的抗药性

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摘要： 为了了解并掌握马铃薯甲虫 *Leptinotarsa decemlineata* (Say)在新疆的抗性水平及动态，为科学用药提供理论依据，利用点滴法测定了新疆北部马铃薯甲虫 6 个田间种群(乌鲁木齐、阜康、昌吉、阿勒泰、尼勒克和特克斯）的 4 龄幼虫对 14 种常规药剂的敏感性，并测试了磷酸三苯酯(TPP)、顺丁烯二酸二乙酯(DEM)和氧化胡椒基丁醚(PBO)的增效作用。结果表明：与已报道的基线相比，特克斯种群对几种药剂都敏感，因此确定特克斯田间种群为敏感品系。与特克斯敏感品系相比，昌吉种群对三氟氯氰菊酯、溴氰菊酯、克百威及丁硫克百威的抗性倍数分别为 108.8, 30, 17.6 和 24.7，而尼勒克种群对这 4 种药剂的抗性倍数分别为 29.4, 42.5, 10.1 和 89.9。此外，昌吉和阜康种群对高效氯氰菊酯也分别产生 8.9 和 13.0 倍的中等抗性。增效测定结果显示：对于尼勒克种群，PBO 对三氟氯氰菊酯有明显增效作用；对于昌吉种群，PBO, TPP 和 DEM 对克百威有明显增效作用。因此推断解毒酶活性增强可能是新疆马铃薯甲虫对三氟氯氰菊酯和克百威的抗性机制之一。由于增效作用不能完全消除抗性，因此新疆马铃薯甲虫的抗药性还可能涉及到其他机制。

关键词： 马铃薯甲虫；抗药性；点滴法；增效剂；增效作用；抗性机制

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